

GNCA-P03-007

49. (new) A method for attenuating expression of a target gene in mammalian cells, comprising introducing into the mammalian cells a single-stranded hairpin RNA comprising self complementary sequences that form a duplex region, which self complementary sequences hybridize under intracellular conditions to a target gene, wherein said hairpin RNA (i) is cleaved in the mammalian cell to produce an RNA guide sequence that enters an Argonaut-containing complex, and (ii) reduces expression of said target gene in a manner dependent on the sequence of said complementary regions.
50. (new) The method of claim 48 or 49, wherein the hairpin RNA is transfected into said mammalian cells.
51. (new) The method of claim 48 or 49, wherein the hairpin RNA is microinjected into said mammalian cells.
52. (new) The method of claim 48 or 49, wherein the hairpin RNA is transcriptional product that is transcribed from an expression construct introduced into said mammalian cells, which expression construct comprises a coding sequence for transcribing said hairpin RNA, operably linked to one or more transcriptional regulatory sequences.
53. (new) The method of claim 52, wherein said transcriptional regulatory sequences include a promoter for an RNA polymerase.
54. (new) The method of claim 53, wherein said transcriptional regulatory sequences include a promoter for a bacteriophage RNA polymerase.
55. (new) The method of claim 53, wherein said transcriptional regulatory sequences include a promoter for an cellular RNA polymerase.
56. (new) The method of claim 53, wherein said promoter is selected from the group consisting of a U6 promoter, a T7 promoter, a T3 promoter, and an SP6 promoter.
57. (new) The method of claim 52, wherein said transcriptional regulatory sequences includes an inducible promoter.
58. (new) The method of claim 52, wherein said mammalian cells are stably transfected with said expression construct.

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59. (new) The method of claim 48 or 49, wherein said hairpin RNA is a transcriptional product of an RNA-dependent RNA polymerase.
60. (new) The method of claim 48 or 49, wherein the cells are germ line cells.
61. (new) The method of claim 60, wherein the cells are stem cells.
62. (new) The method of claim 48 or 49, wherein the cells are somatic cells.
63. (new) The method of claim 48 or 49, wherein the cells are immortalized cells.
64. (new) The method of claim 48 or 49, wherein the mammalian cells are primate cells.
65. (new) The method of claim 64, wherein the primate cells are human cells.
66. (new) The method of claim 48 or 49, wherein the mammalian cells are selected from the group consisting of adipocytes, fibroblasts, myocytes, cardiomyocytes, endothelium, neurons, glia, blood cells, megakaryocytes, lymphocytes, macrophages, neutrophils, eosinophils, basophils, mast cells, leukocytes, granulocytes, keratinocytes, chondrocytes, osteoblasts, osteoclasts, hepatocytes, and cells of the endocrine or exocrine glands.
67. (new) The method of claim 48 or 49, wherein the hairpin RNA is introduced into the mammalian cells in cell culture.
68. (new) The method of claim 48 or 49, wherein the hairpin RNA is introduced into the mammalian cells in an animal.
69. (new) The method of claim 48 or 49, wherein expression of the target is attenuated by at least 33 percent relative expression in cells not treated said hairpin RNA.
70. (new) The method of claim 48 or 49, wherein expression of the target is attenuated by at least 90 percent relative expression in cells not treated said hairpin RNA.
71. (new) The method of claim 48 or 49, wherein the target gene is an endogenous gene of the mammalian cell.

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72. (new) The method of claim 48 or 49, wherein the target gene is a heterologous gene relative to the genome of the mammalian cell.
73. (new) The method of claim 48 or 49, wherein the target gene is a gene of a pathogen.
74. (new) The method of claim 48 or 49, wherein the self complementary sequences hybridize under intracellular conditions to a mature mRNA transcript.
75. (new) The method of claim 48 or 49, wherein the self complementary sequences hybridize under intracellular conditions to a non-coding sequence of the target gene.
76. (new) The method of claim 48 or 49, wherein the self complementary sequences hybridize under intracellular conditions to an untranscribed sequence of the target gene, which untranscribed sequence is operably linked to the coding sequence of the target gene.
77. (new) The method of claim 48 or 49, wherein the self complementary sequences hybridize under intracellular conditions to a non-coding sequence of the target gene selected from the group consisting of promoter sequence, enhancer sequence and intronic sequence.
78. (new) The method of claim 48 or 49, wherein the self complementary sequences hybridize under intracellular conditions to a target gene selected from the group consisting of developmental genes, oncogenes, tumor suppressor genes, and genes encoding enzymes.
79. (new) The method of claim 48 or 49, wherein the hairpin RNA includes one or more modifications to phosphate-sugar backbone or nucleosides residues.
80. (new) The method of claim 79, wherein the modifications inhibit inactivation of the hairpin RNA by adenosine deaminase.
81. (new) The method of claim 48 or 49, wherein the self complementary sequences are 20-50 nucleotides in length.
82. (new) The method of claim 48 or 49, wherein the self complementary sequences are 29 nucleotides in length.

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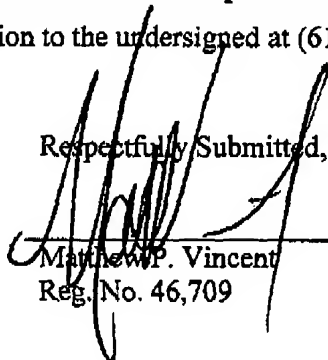
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83. (new) A method for attenuating expression of one or more target genes in mammalian cells, comprising introducing into the mammalian cells a variegated library of single-stranded hairpin RNA species, each hairpin RNA species comprising self complementary sequences that form duplex regions and which hybridize under intracellular conditions to a target gene, wherein each of said hairpin RNA species (i) is a substrate for cleavage by a RNaseIII enzyme to produce a double-stranded RNA product, and (ii) reduces expression of a target gene in a manner dependent on the sequence of said complementary regions.
84. (new) The method of claim 83, wherein said variegated library of hairpin RNA species collectively attenuate expression of a plurality of different target genes.
85. (new) The method of claim 83, wherein said variegated library of hairpin RNA species are arrayed a solid substrate.
86. (new) The method of claim 83, wherein said variegated library of hairpin RNA species are arrayed in wells of a multi-well plate.
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Applicants believe no fees are due, however, if there are any fees due in connection with the filing of this Second Preliminary Amendment, please charge the fees to our **Deposit Account No. 18-1945**. Please direct any questions arising from this submission to the undersigned at (617) 951-7000.

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Respectfully Submitted,


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